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Sensorimotor Gating and D₂ Receptor Signalling: Evidence from a Molecular Genetic Approach

Short Title: Sensorimotor Gating and D₂ Receptor Signalling

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Abstract

Converging evidence from pharmacological investigations, genetic association studies and schizophrenia research indicates an important influence of the dopamine system on sensorimotor gating as measured by prepulse inhibition (PPI) of the acoustic startle response. In particular, D_2 receptor agonists have been shown to disrupt PPI in humans and rodents. In the present study, we investigated the associations of two functional *DRD2* related single nucleotide polymorphisms (rs4648317 and rs1800497, the latter also known as *DRD2* / *ANKK1* Taq1A) with PPI in two independent healthy human samples (overall N=197; Munich N=101; London N=96). Taq1A is a prominent marker of striatal D_2 receptor signalling and was therefore hypothesized to impact on PPI. In line with our hypothesis, we report here reduced PPI levels in individuals with higher striatal D_2 receptor signalling as indicated by the Taq1A genotype. Meta-analysis across both samples confirmed this finding. In contrast, an association between rs4648317 and PPI found in the Munich sample could not be confirmed in the London sample. Overall, the present study helps to bridge the gap between pharmacological manipulations of PPI and molecular genetics of the dopaminergic system.

Keywords:

Prepulse Inhibition, Dopamine, Acoustic Startle Response, *DRD2*, Taq1a

1. Introduction

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is a widely used psychophysiological measure of sensorimotor gating (Braff et al., 1978, Geyer et al., 1990, Swerdlow and Geyer, 1998) which helps to avoid sensory information overflow (Braff et al., 1999). In this paradigm, in humans first described by Krauter (1973) and systematically investigated by Graham (1975), a loud noise is used to produce a startle reflex that is commonly measured electromyographically above the *Musculus orbicularis oculi* as an eyeblink reflex. Graham (1975) found that a non-startling prestimulus preceding the startling stimulus by 30-500msec reduces the eyeblink reflex of human subjects.

PPI is reduced in people with schizophrenia (e.g., Braff et al., 1978, Kumari et al., 2008, Swerdlow et al., 2006), in unaffected first-degree relatives of schizophrenia patients (Cadenhead et al., 2000, Kumari et al., 2005b), in the prodromal stage of schizophrenia (Quednow et al., 2008) and in schizotypal personality disorder (SPD) (Cadenhead et al., 1993, Cadenhead et al., 2000). PPI is partially heritable (Anokhin et al., 2003, Dulawa and Geyer, 2000, Greenwood et al., 2007, Willott et al., 2003) with heritability estimates ranging from 32% to >50%. Together, these studies indicate that PPI may represent a schizophrenia endophenotype (Gottesman and Gould, 2003).

The neural basis of PPI in rodents lies in the cortico-pallido-striatal-thalamic and pontine circuitry, with evidence for involvement of the prefrontal cortex, thalamus, hippocampus, nucleus accumbens (NAC), striatum, ventral pallidum (VP), globus pallidus, and subpallidal efferents to the pedunculo-pontine nucleus (PPTg) (review, Swerdlow et al., 2008). Functional (Campbell et al., 2007, Kumari et al., 2003, Kumari et al., 2007, Neuner et al., 2010) and structural (Kumari et al., 2005a) neuroimaging studies agree with involvement of these regions in human PPI.

Important clues in the ongoing investigation of the neural control of PPI come from pharmacological studies. Specifically, there is evidence for an important role of the D_2 dopamine receptor (DRD2). For example, it has been shown that DRD2-agonists in rats disrupt PPI (Peng et al., 1990, Swerdlow et al., 2002b). Furthermore, PPI deficits induced by apomorphine (a D_1 and D_2 receptor agonist) in rats can be reversed by haloperidol (which acts as a D_2 antagonist) exhibiting a dose-response relation that is highly correlated with its antipsychotic potency in schizophrenia patients (Martinez et al., 2000, Swerdlow et al., 1994, Swerdlow and Geyer, 1993). These disruptive effects of D_2 receptor agonists on PPI are presumably mediated by dopaminergic activity in the NAC (review, Swerdlow et al., 2001): increased dopaminergic signaling in the NAC is thought to reduce activity of GABAergic projections to the VP (Kretschmer and Koch, 1998). VP activity, in turn, is assumed to cause a tonic regulation of the PPTg which is thought to mediate PPI via innervations of the nucleus reticularis pontis caudalis (PnC) (review, Fendt et al., 2001). Irrespective of NAC activity, the caudate nucleus also seems to play a role in the dopaminergic regulation of PPI (Hart et al., 1998).

In contrast to rats, in humans the effects of D_2 receptor agonists such as bromocriptine are less clear: whereas some studies found a disruptive effect of bromocriptine on PPI (Abduljawad et al., 1998, Abduljawad et al., 1999), others did not (Swerdlow et al., 2002a, 2002b).

Complementary to pharmacological manipulation studies, the investigation of functional genetic polymorphisms offers another way to elucidate the role of specific neurotransmitter systems in human PPI. An advantage of this approach is that naturally occurring variation in the expression level or functional properties of a protein is used without exogenous manipulations as in pharmacological investigations. Focusing on dopaminergic influences on sensorimotor gating such studies have established associations between a number of genetic polymorphisms and PPI. Among the most prominent single nucleotide polymorphisms (SNP) showing association with PPI are the *catechol-O-methyltransferase* (COMT) Val158Met SNP (Quednow et al., 2009,

2010, Roussos et al., 2008b), and the *dopamine-receptor D3 (DRD3)* Ser9Gly SNP (Roussos et al., 2008a). These molecular genetic findings strengthen the evidence for a modulatory role of dopamine in PPI.

Accordingly, in order to further probe the role of the *D*₂ receptor in PPI, some previous studies have examined associations of PPI with SNPs in the *D*₂ dopamine receptor (*DRD2*) gene (Hokyo et al., 2010, Montag et al., 2008, Roussos et al., 2008b). Hokyo and colleagues (2010) investigated interaction effects between Japanese schizophrenia patients and healthy controls on the association between PPI and *DRD2* rs1801028 and rs6277, respectively. Montag et al. (2008) and Roussos et al. (2008b) examined the *DRD2/ANKK1* Taq1A (rs1800497) SNP in healthy, Caucasian subjects. None of these studies obtained a significant association between the *DRD2* SNPs and PPI. However, the two studies of the *DRD2/ANKK1* Taq1A SNP (Montag et al. 2008, Roussos et al. 2008b), a polymorphism widely studied in the context of striatal dopamine signaling, used somewhat uncommon startle parameters and experimental variations of prepulse intensity, respectively. Importantly, small changes in stimulus characteristics can considerably affect PPI (e.g., Csomor et al., 2006, 2008, Hsieh et al., 2006). Therefore, the generalisability of those initial *DRD2/ANKK1* Taq1A SNP findings is limited. Additionally, Montag et al. (2008) sampled only female subjects, whereas Roussos (2008b) included only male subjects.

Given the role of the *DRD2/ANKK1* Taq1A SNP in striatal dopamine signaling (Hirvonen et al., 2009, Jönsson et al., 1999, Pohjalainen et al., 1998a, Ritchie and Noble, 2003, Thompson et al., 1997), it is important to further examine this matter using established stimulus characteristics with known sensitivity to schizophrenia (Kumari and Ettinger, 2005). The present study therefore aims to clarify the influence of *D*₂ receptor signalling on sensorimotor gating by investigating associations of *DRD2* SNPs with PPI. Specifically, two functional *DRD2* related SNPs, rs7122454/rs4648317 and rs1800497 (*DRD2/ANKK1* Taq1A), are examined.

The *DRD2/ANKK1* Taq1A polymorphism is located 9.5kb downstream from the *DRD2* gene (Grandy et al., 1993) in the *ankyrin repeat and kinase domain containing 1* (*ANKK1*) gene. The *ANKK1* and *DRD2* genes overlap and share haplotype blocks. The Taq1A SNP leads to a substitution of an acidic amino acid for a basic amino acid (Glu713Lys) which potentially might lead to a change in protein function. The mutated A1 allele has a prevalence of 19.5%, the homozygous A1A1 genotype of 5.3% in healthy humans of Caucasian descent (The International HapMap Consortium, 2003). Therefore, the A1 allele carriers are often contrasted with carriers without the A1 allele, i.e. A2 allele homozygotes. Crucially, several positron emission tomography and autoradiographic studies have shown that the minor A1 allele is associated with reduced striatal D_2 receptor density (up to 30%) and receptor binding compared to the A2A2 allele status in humans (Hirvonen et al., 2009, Jönsson et al., 1999, Pohjalainen et al., 1998a, Ritchie and Noble, 2003, Thompson et al., 1997). In addition, the A1 allele has been associated with reduced substantia nigra volume (Cerasa et al., 2009), a key dopaminergic area. The role of the Taq1A polymorphism in the aetiology of schizophrenia is controversial: whereas some case-control studies found an association of the A1 allele with schizophrenia (Comings et al., 1991, Monakhov et al., 2008), other studies provided evidence for an increased frequency of the A2 allele in schizophrenia patients (Dubertret et al., 2001, 2004, 2010, Vijayan et al., 2007). Golimbet et al. (2003) found that schizophrenia-concordant sibling pairs had significantly more often common alleles at the Taq1A locus.

The second SNP investigated in this study is rs4648317/rs7122454, two SNPs in complete linkage disequilibrium (LD). This SNP (unlike Taq1A) has been associated in females with prolactin concentrations (Fukui et al., 2010). Prolactin secretion is inhibited by the hypothalamic dopamine transmission via the D_2 receptor (Grattan and Kokay, 2008). Specifically, the T allele was associated with increased prolactin concentration presumably caused by a reduced D_2 receptor transmission. This SNP is located in intron 1 of *DRD2* and alters two potential transcription factor binding sites

(Laucht et al., 2008) which might indicate an effect on *DRD2* expression. Laucht and colleagues (2008) also reported an association between rs4648317 and nicotine dependence in adolescents, with the T allele carrier status related to higher nicotine dependence. As nicotine causes striatal dopamine release in rats (e.g. Kaiser et al., 1998, Wonnacott et al., 2000) and in humans (Brody et al., 2004), Laucht et al. (2008) suggested that the association between *DRD2* SNPs and nicotine dependence might rely on changes in striatal *DRD2* expression levels that influence, in turn, nicotine-induced reward mediated by dopamine release. If this is true, we would also expect an association of this SNP with PPI.

In addition, Hamidovic and colleagues (2009) found an association between rs4648317 genotype and inhibitory performance in the stop signal task as well as self-report impulsivity (Zuckerman–Kuhlman Personality Questionnaire, Impulsivity subscale). Both measures indicated lower impulsivity of T allele carriers compared to CC carriers.

In summary, we investigated the association with PPI of two *DRD2* SNPs thought to influence striatal dopamine signalling. Given the importance of replication in molecular genetics (Chanock et al., 2007) we investigate this relationship in two independent samples. Based on previous molecular studies, we hypothesized that Taq1A A2A2 allele carriers, who exhibit higher striatal *DRD2* levels compared to A1 carriers, would show reduced PPI. Considering rs4648317/rs7122454, we had no directed hypothesis. However, based on previous research on nicotine dependence we expected an association between this SNP and PPI.

2. Methods and Materials

2.1 Participants

2.1.1 Munich sample

One hundred eighteen healthy unrelated volunteers of Caucasian descent were recruited through local advertisements in Munich, Germany. Exclusion criteria consisted of (1) any current psychiatric diagnosis (using the German version of the Mini-International Neuropsychiatric Interview; Ackenheil et al., 1999), (2) a history of neurological complications, (3) a history of psychosis or attention deficit hyperactivity disorder in first-degree relatives, (4) any current physical condition, (5) any current consumption of over-the-counter or prescription medication (except for contraceptives), and (6) any hearing impairments or tinnitus. Participants provided demographic information and data on smoking status and severity (using the Fagerström test for nicotine dependence; Heatherton et al., 1991). Subjects scoring >0 on this test were considered smokers.

2.1.2 London sample

One hundred and six healthy Caucasian volunteers were recruited through local advertisements in South London, UK. Exclusion criteria consisted of (1) DSM-IV Axis I disorders using the Structured Clinical Interview for DSM-IV Disorders for healthy subjects (SCID-NP), (2) a history of neurological complications, substance abuse or dependence, or hearing impairments, (3) a history of psychosis in first-degree relatives, and (4) any current physical condition or medication. The assessment of smoking behaviour was identical to the Munich sample.

For both samples, the study had approval by the local ethics committees. Subjects provided written informed consent and were reimbursed for their participation.

2.2 Genotyping

For the Munich sample DNA was extracted from 3 ml saliva samples using QIAamp DNA-Blood-Midi-Kit (QIAGEN, Germany). For the London sample DNA was extracted from cheek swabs or venous blood. Genotyping was based on MALDI- (*Matrix Assisted*

Laser Desorption/Ionisation) TOF (*time of flight*) mass spectrometry (Haff and Smirnov, 1997). Therefore, genomic sequences flanking the SNPs of interest of the extracted DNA samples were first amplified using a Multiplex *polymerase chain reaction* (PCR), otherwise called *SNP Capture* reaction (Oeth et al., 2005). The primers for the Multiplex PCR were as follows: for *ANKK1* Taq1A (rs1800497) the forward primer: 5'-XTG TGC AGC TCA CTC CAT CCT-3' and the reverse primer: 5'-XTC AAG GGC AAC ACA GCC ATC-3'; for *DRD2* rs7122454 the forward primer: 5'-XTA AAA TGG TGG TGG CCA GAG-3' and the reverse primer: 5'-XGT CTT GAA ACA CTG AAG CTC-3' (X=ACGTTGGATG; *mass tag*).

MassExtend-primers that were hybridizing at the 5' end directly in front of the polymorphic locus of the PCR products were used for genotyping. These MassExtend-primers (including possible extensions) were as follows: for *ANKK1* Taq1A (rs1800497): 5'-CAT CCT CAA AGT GCT GGT C(A/G)-3'; for *DRD2* rs7122454: 5'-ACG AAT TCT CCA TGT TAC T(C/G)-3'. In the cyclic iPLEX reaction the MassExtend primers were extended by a single base (*Single Base Extension*, SBE) that was complementary to the base at the polymorphic locus. These SBE products were then analyzed by MALDI-TOF mass spectrometry (Oeth et al., 2005). Thereby, the SNP genotype could be identified.

Genotyping was successful in 98.1% of subjects for both investigated SNPs in the Munich sample. For the London sample Genotyping was successful in 97.1% of subjects for the rs1800497 SNP and 94.2% of subjects for the rs7122454 SNP.

2.3 Startle Response Measurement

The acoustic startle response (ASR) was measured with a commercially available electromyographic (EMG) startle reflex system (SR-Lab, San Diego, CA). The system generates and delivers the acoustic stimuli and, records and scores EMG activity for a period of 250 msec (sample interval = 1 msec) beginning at the onset of the acoustic

stimulus. The stimuli were presented binaurally to the subjects via headphones while they were sitting comfortably in a moderately lit laboratory room. The startle response was recorded from the right *orbicularis oculi* muscle with two (6 mm) silver/silver chloride electrodes filled with electrode gel. The electrodes were placed approximately 1 cm below the pupil and 1 cm below the outer corner of the right eye. The ground electrode was located behind the left ear above the mastoid. Previously, the skin at these positions was cleaned with sterile alcohol swabs.

Each session started with a 4-min acclimatization period consisting of 70dB white noise that was present throughout the session. Subjects were then presented with 49 white-noise sound pulses. Each pulse-alone (PA) stimulus had an intensity of 110dB in the Munich sample and 115dB in the London sample which was presented for a period of 40msec. The inter-trial intervals varied between 9 and 23sec (mean=15sec). In 36 of these trials, there was an 85-dB white noise prepulse presented for a period of 20msec preceding the pulse with a stimulus-onset asynchrony (SOA) of 30, 60 or 120msec (12 trials for each SOA). The first PA trial was not used in the statistical analysis. The order of the remaining 48 trials was pseudo-randomized. The whole session lasted approximately 16 minutes.

Each trial was inspected offline in order to exclude unusable trials, i.e. trials with unstable or noisy data. Scoring was performed by the analytic SR-Lab program. The criterion for the onset latency (msec) was a shift of 6 digital units from the baseline value occurring in a time window of 21–120msec after the pulse. The baseline was determined by the mean amplitude (arbitrary analogue-to-digital units) in the first 20msec after the pulse stimulus. The peak latency (msec) was defined as the point in time of maximal magnitude occurring within 150msec after the acoustic stimulus.

Exclusion criteria for single trials were periods of more than 95msec between the onset and peak latencies, and onsets latency larger than 100msec. For all subjects the amplifier gain control was kept constant. A 50-Hz band-pass filter was applied to eliminate interference from the power mains. Subjects with response rejections > 30%

in PA trials were excluded from further analysis. Smoking before the assessment was not restricted to prevent effects of smoking withdrawal (Kumari et al., 1996).

2.4 Statistical Analysis

Statistical analysis using SPSS 17.0 (SPSS Inc., Chicago, IL) was identical in both samples. The significance level was set at $p < .05$. P-values between .1 and .05 were considered trends towards significance. In order to eliminate individual differences in startle reactivity, percent PPI was calculated as follows:

$$\%PPI = 100 \cdot \frac{(\text{Amplitude of pulse-alone trial}) - (\text{Amplitude of prepulse trial})}{\text{Amplitude of pulse-alone trial}}$$

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PPI was computed for

each SOA and subject separately. Previous research has shown that gender (Aasen et al., 2005, Swerdlow et al., 1993) and smoking status (Swerdlow et al., 2006) influences PPI. Therefore, percent PPI was analyzed by analysis of covariance (ANCOVA) using the factors SOA and genotype of both SNPs and the covariates gender and smoking status.

For the analysis of the habituation of the ASR, PA trials were divided into four blocks. Percent habituation was calculated as percent reduction in startle magnitude between the first and second block as well as between the first and last block of PA trials. Startle reactivity was measured by calculating the mean values of PA trials in the first block and across all four blocks. Moreover, the mean magnitudes of prepulse trials and latencies to onset and peak of the ASR in PA trials were analyzed.

The analyses of genotype associations with demographic data and psychophysiological measures (habituation, startle reactivity and latency to onset) were conducted using one-way ANOVAs for continuous measures and Chi-square tests for

frequency data. Greenhouse-Geisser correction of degrees of freedom was applied to ANOVAs if the assumption of sphericity was violated as assessed by Mauchly's test. On the basis of significant interactions post-hoc pairwise t-tests using Bonferroni correction were conducted. As the distribution of startle magnitudes significantly deviated from normal **distribution** a square root transformation was applied to normalize the data. It should be noted that the pattern of significant findings was independent of whether square root transformed or untransformed variables were used.

To estimate effect sizes for between-group differences we used Cohen's d. These calculations are based on pooled SOA conditions. To estimate effect sizes for differences between more than two groups partial eta-squared values were calculated (Pierce et al., 2004).

Finally, in order to provide summary statistics of genotype associations across the two samples in this study, meta-analysis was carried out to assess genotype associations with PPI across both samples by using Stouffer's z-trend method and Fisher's trend to combine p-values (Whitlock, 2005). For the analysis of effect sized across samples a random effects model was used according to Hedges and Olkin (1985).

3. Results

3.1 Munich sample

Fifteen subjects were startle non-responders as defined by response rejections in more than 30% of PA trials and were excluded from further analysis, leaving a total sample of N=101.

As expected, rs7122454 and rs4648317 were in complete LD ($r^2=1.0$, $d'=1.0$). *ANKK1* Taq1A (rs1800497) and rs7122454/rs4648317 polymorphisms were not in strong LD ($r^2=0.01$; $d'=0.57$). For *ANKK1* Taq1A ($\chi^2(1)=0.48$; $p=.49$) and rs7122454/rs4648317

($\chi^2(1)=0.67$; $p=.41$) genotype frequencies were distributed in accordance to the Hardy-Weinberg Equilibrium (HWE).

3.1.1 Genetic Associations with PPI

Demographic and startle data of the Munich sample and their distribution across genotype groups are shown in Table 1 (for habituation and latency data see Supplementary Table S1). The *ANKK1* Taq1A genotype groups differed in the gender distribution. However, startle magnitudes did not differ significantly between genotype groups.

PPI was affected by genotype: a 3x2x2 (SOA condition x *DRD2* rs7122454 SNP x *ANKK1* Taq1A SNP) repeated measures ANCOVA with the covariates gender and smoking status revealed a significant main effect of SOA ($F[2,190]=12.8$; $p<.001$; partial $\eta^2=.12$) and a trend-level effect of the covariate gender reflecting stronger PPI in males than females ($F[1,95]=3.4$; $p=.07$; partial $\eta^2=.04$). Moreover, there was an interaction between SOA and *ANKK1* Taq1A genotype ($F[2,190]=5.9$; $p<.01$; partial $\eta^2=.06$) and a significant main effect of Taq1A genotype ($F[1,95]=4.3$; $p<.05$; partial $\eta^2=.04$; $d=.236$), reflecting reduced PPI for A2A2 allele carriers compared to A1 allele carriers. In addition, there was a trend towards significance for rs7122454 ($F[1,95]=3.5$; $p=.07$; partial $\eta^2=.04$; $d=.660$) pointing to lower PPI in CG/GG allele carriers compared to CC allele carriers. Finally, a significant interaction between the two SNPs was found ($F[1,95]=5.3$; $p<.05$; partial $\eta^2=.05$) (see Fig 1). Bonferroni-corrected t-tests showed that subjects with the combination A2A2/rs7122454 G had significantly reduced PPI compared to A2A2/rs7122454 CC ($p<.001$; $d=.872$) and to A1/rs7122454 G ($p<.05$; $d=.775$) carriers. Furthermore, post-hoc tests on the basis of the significant interaction between SOA and *ANKK1* Taq1A genotype revealed that A2A2 carriers had reduced PPI only at SOA 120msec ($p<.01$; $d=.590$) and by trend at SOA 60msec ($p=.06$;

$d=.162$). At SOA 30msec, there was no significant difference between the Taq1A genotype groups ($p>.5$).

Finally, the effects of gender on the main effects of the two polymorphisms were investigated: when restricting the analysis to female subjects we observed a trend towards a main effect of Taq1A ($F[1,65]=3.8$; $p=.056$; $d=.264$; partial $\eta^2=.06$) and a significant effect of rs7122454 ($F[1,65]=4.5$; $p<.05$; $d=.726$; partial $\eta^2=.06$). In contrast, when analyzing only male subjects there was no significant association between PPI and Taq1A ($F[1,26]=1.0$; $p=.32$; $d=.786$; partial $\eta^2=.04$) or rs7122454 ($F[1,26]=0.2$; $p=.63$; $d=.562$; partial $\eta^2=.01$).

3.2 London sample

Four subjects were startle non-responders and excluded from further analysis, leaving a final sample of $N=96$.

As expected, rs7122454 and rs4648317 were in complete LD ($r^2=1.0$, $d'=1.0$). Again, *ANKK1* Taq1A (rs1800497) and rs7122454/rs4648317 were in weak LD ($r^2=0.002$; $d'=0.30$). For *ANKK1* Taq1A ($\chi^2(1)=0.12$; $p=.73$) and rs7122454/rs4648317 ($\chi^2(1)=0.06$; $p=.80$) genotype frequencies were distributed in accordance to HWE.

3.2.1 Genetic Associations with PPI

Demographic and startle data of the London sample and their distribution across genotype groups are shown in Table 2 (for habituation and latency data see Supplementary Table S2). The *DRD2* rs7122454 genotype groups differed in their gender distribution. However, startle magnitudes did not differ significantly between genotype groups.

PPI was associated with one of the genotypes: a 3x2x2 (SOA condition x *DRD2* rs7122454 SNP x *ANKK1* Taq1A SNP) repeated measures ANCOVA with the covariates gender and smoking status revealed a significant main effect of Taq1A

genotype ($F[1,90]=7.2$; $p<.01$; partial $\eta^2=.07$; $d=.752$). This finding reflects reduced PPI for A2A2 allele carriers compared to A1 allele carriers (see Fig 2). In contrast, there was no effect of rs7122454 genotype ($F[1,90]=0.6$; $p=.44$; partial $\eta^2=.01$; $d=.002$) or an interaction between the two SNPs ($F[1,90]=0.1$; $p=.80$; partial $\eta^2=.001$). Additionally, there was the expected significant main effect of SOA ($F[2,180]=7.7$; $p<.01$; partial $\eta^2=.08$). Finally, there was a significant effect of the covariate gender ($F[1,90]=12.3$; $p<.01$; partial $\eta^2=.12$) indicating stronger PPI in males than females.

Next, potential effects of gender on the Taq1A main effect were investigated: when restricting the analysis of the current data to female subjects we still observed a significant main effect of Taq1A ($F[1,42]=4.6$; $p<.05$; $d=.704$; partial $\eta^2=.06$). In contrast, when analyzing only male subjects there was no significant association between PPI and Taq1A ($F[1,44]=2.9$; $p=.10$; $d=.508$; partial $\eta^2=.06$).

3.3 Meta-Analysis

Meta-analysis across the two samples confirmed a significant effect of Taq1A ($p<.01$). Using the random effects model the combined effect size was $d=.49$.

For rs7122454 the combined p-value was not statistically significant ($p>.1$). Using Fisher's trend method the meta-analysis of the interaction between both investigated SNPs reveals a trend towards significance ($p=.06$) across samples.

4. Discussion

In the present study we aimed to investigate the relationship between sensorimotor gating and striatal *D*₂ receptor signalling via two functional *DRD2* related polymorphisms. Our results indicate an influence of *DRD2* genotype on PPI: in the Munich sample both SNPs were associated with PPI. Considering the Taq1A SNP, the A2A2 allele status was associated with reduced PPI. This effect was replicated in the

London sample. With regard to rs7122454 (rs4648317), G (T) allele carriers showed reduced PPI in the initial sample. However, this effect was not confirmed by the data obtained from the London sample. Meta-analysis across both samples indicated a significant association of Taq1A but not of rs4648317/rs7122454 with PPI. In addition, we found a trend for an interaction between both SNPs across the two samples.

Dopaminergic neurotransmission plays a major role within the striatum (e.g. Schultz, 2002) and there is consistent evidence of striatal involvement in PPI modulation (review, Swerdlow et al., 2008). Some human pharmacological studies too, as noted earlier (Introduction), reveal the impact of striatal, dopaminergic neurotransmission on PPI (Abduljawad et al., 1998, 1999). In rats, increased dopaminergic neurotransmission in the NAC disrupts PPI via projections from the VP to the PPTg (Kretschmer and Koch, 1998). The present results are in line with these findings: the Taq1A A2A2 allele status that has previously been associated with increased striatal D_2 receptor availability (Jönsson et al., 1999, Pohjalainen et al., 1998a, Ritchie and Noble, 2003, Thompson et al., 1997) shows an association with reduced PPI in our sample. The association of the Taq1A SNP with PPI is likely due to increased striatal D_2 receptor availability in A2A2 allele carriers compared to A1 allele carriers.

The present findings can also be reconciled with evidence from dopaminergic disorders such as schizophrenia. Interestingly, it has been shown that in schizophrenia patients there is an increased baseline occupancy of striatal D_2 receptors (Abi-Dargham et al., 2000). Moreover, unaffected monozygotic twins of schizophrenia patients exhibit increased D_2 receptor density in caudate compared with unaffected dizygotic twins and healthy controls (Hirvonen et al., 2005). Together with the current results, these findings suggest that increased striatal D_2 dependent dopamine signalling might be at least partially responsible for PPI deficits in schizophrenia patients and their relatives. Future studies combining molecular imaging of striatal D_2 receptor signalling, PPI recordings, and molecular genetics will allow to critically evaluate this hypothesis.

In the Munich sample, the association of Taq1A with PPI was present for the SOAs of 60 and 120msec but not for 30msec. At longer SOAs attentional processes can modulate PPI (Filion et al., 1993). As these modulatory influences are not present at very short SOAs, D_2 receptor dependent neurotransmission might not affect PPI at those SOAs which would explain the present findings. Earlier Kumari and colleagues (1999) had suggested a particularly strong influence of dopaminergic (D_2) status on PPI at longer SOAs based on their data showing normal range 120-msec PPI in patients medicated with typical or atypical antipsychotics whereas only clozapine appeared to be effective at shorter SOAs.

In contrast to the present results, Montag et al. (2008) and Roussos et al. (2008b) did not find associations of Taq1A SNP with PPI. The discrepancy of our findings and these previous results might be due to differences in the PPI measurement and sample characteristics: PPI was measured by Montag and colleagues using intensities of background noise, prepulse and pulse of 55dB, 74dB and 106dB, respectively. In contrast, in the current study, the intensity of background noise, prepulse and pulse was at 70, 85 and 110/115dB, respectively. We chose these parameters because of their proven sensitivity to schizophrenia in many previous studies (Kumari and Ettinger, 2005). It has been already shown that stimulus characteristics (e.g. ambient 54 dB background noise versus experimentally delivered 70 dB background noise) dramatically alter PPI in healthy people (Hsieh et al., 2006) and can produce divergent results in comparisons of schizophrenia versus healthy populations (Braff et al., 2001). It is likely that stimulus characteristics are also a source of variance in genetic studies of human PPI. The sample of Roussos et al. included only young males and the startle paradigm used an experimental variation of the loudness of the prepulse. The two samples used in the current study were of mixed gender and demographically more heterogeneous. However, when we analyzed the gene effects for each gender separately we still found an association between Taq1A and PPI in female subjects. In contrast, no such effect was found in male subjects. This suggests that the association

found in the current study is mainly driven by female subjects which could explain the lack of an association of the Taq1A polymorphism with PPI in the Roussos study. It is likely that the absence of an association between Taq1A and PPI in the Munich sample is due to the small number of male subjects and to the interaction between gender and Taq1A genotype (the reasons for which are difficult to explain and currently unknown) yielding only very few male subjects with A1 genotype (N=3). However, in the London sample there are 18 male subjects with A1 genotype (and 15 female A1 subjects) and still there was no significant effect of Taq1A on PPI in male subjects. Whereas previous imaging studies on the effects of Taq1A on striatal D_2 receptor signaling have not found gender differences (Jönsson et al., 1999, Pohjalainen et al., 1998a) the present results might be due to the generally lower striatal D_2 receptor affinity found in women compared to men (Pohjalainen et al., 1998b). Higher D_2 receptor affinity in men might mask the effects of Taq1A via changes in the D_2 receptor density on PPI.

Finally, on a descriptive level, Montag and colleagues (2008) also found lower levels of PPI for female A2A2 allele carriers compared to A1 carriers.

Considering the second SNP investigated here, rs7122454 (rs4648317), the results of the Munich sample indicated that the G(T) allele status might be associated with reduced PPI. Previous research has shown that the G(T) allele status might also be related to increased nicotine dependence (Laucht et al., 2008). Laucht and colleagues suggested that this finding might rely on changes in striatal *DRD2* expression which affects in turn the nicotine-induced reward. Interestingly, high levels of nicotine dependence have also been shown to disrupt PPI under smoking withdrawal (Kumari and Gray, 1999). The present relation might therefore be due to increased striatal D_2 receptor availability for the rs7122454 G/rs4648317 T genotype. However, since this effect could not be replicated in the London sample and imaging data are missing for this SNP, this conclusion remains speculative and requires further investigation.

The differences in %PPI between the current samples require further discussion. Apart from population differences between London and Munich there are differences in

stimulus characteristics across samples: in the Munich sample we used a pulse intensity of 110 db, in contrast to 115db in the London sample. Recently, it has been shown that PPI magnitude is decreasing with increasingly intense pulse stimuli (Csomor et al., 2006, 2008, Yee et al., 2005). The current results confirm these findings: the mean %PPI level (across different SOAs) was significantly lower in the London sample ($30.3\% \pm 17.6$) than in the Munich sample ($44.8\% \pm 21.1$) ($t[195]=5.2$; $p<.001$). As already stated above such differences in stimulus characteristics are a source of additional variance that exacerbates the replication of genetic association studies. This additional variance might also be a reason for the failure to replicate the association between rs7122454 and PPI in the London sample.

In summary, the present results indicate an association of *DRD2* genotype with PPI in healthy humans. Specifically, increased striatal receptor availability as indicated by the Taq1A A2A2 genotype was associated with reduced PPI. This effect was replicated in a second, independent sample. However, the present findings must be considered within the broader context of previous studies, which did not find such an association. Overall, the molecular genetic approach may provide an important contribution to the characterization of the neural mechanisms of PPI by bridging the gap between evidence from pharmacological studies, imaging studies and molecular genetics.

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Statement of Interest

The authors declare no conflict of interest.

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Table 1. Demographic and psychophysiological data of the Munich sample grouped according to *ANKK1* Taq1A and *DRD2* rs7122454 genotype.

<i>ANKK1</i> Taq1A	<i>A2A2</i>		<i>A1</i>		Total	<i>ANKK1</i> Taq1A		<i>DRD2</i> rs7122454		<i>ANKK1</i> Taq1A x <i>DRD2</i> rs7122454		
<i>DRD2</i> rs7122454	CC	CG/GG	CC	CG/GG		F/ χ^2	p	F/ χ^2	p	F/ χ^2	p	df/df _{er}
N	49(49%)	26(25%)	18(18%)	8(8%)	101(100%)							
Age, years	24.3±4.7	26.3±6.3	26.1±6.3	23.8±3.2	24.9±5.4	0.47	0.50	0.08	0.79	1.61	0.21	1/97
Men, %	34.7	42.3	11.1	12.5	30.7	6.04	0.03*	0.51	0.50			1
Smokers, %	8.0	3.8	11.1	0.0	6.9	0.04	1.00	1.23	0.42			1
Years of Education	15.6 ±1.6	16.9±2.6	15.6±2.0	15.9±2.0	16.0±2.0	1.04	0.31	2.71	0.10	0.76	0.39	1/95
First Block, Magnitude of PA Trials (Arbitrary Units)	197.9±132.3	214.2±129.3	237.6±133.6	263.2±189.8	214.3±136.2	1.76	0.19	0.39	0.53	0.02	0.89	1/97
Mean Magnitude of PA Trials (Arbitrary Units)	143.3±101.8	152.5±95.5	180.8±107.1	190.9±120.0	156.2±102.5	2.20	0.14	0.10	0.75	0.00	0.95	1/97
Mean Magnitude of PP30 Trials (Arbitrary Units)	99.4 ±81.7	119.2 ±96.6	145.1 ±99.5	157.0 ±144.4	117.2 ±95.4	2.82	0.10 ^(*)	0.26	0.61	0.17	0.69	1/97
Mean Magnitude of PP60 Trials (Arbitrary Units)	57.1 ±40.2	93.2 ±82.4	86.7 ±74.4	94.5 ±90.3	74.6 ±65.4	0.73	0.39	1.64	0.20	1.25	0.27	1/97
Mean Magnitude of PP120 Trials (Arbitrary Units)	49.9 ±43.8	78.8 ±64.2	53.6 ±59.1	57.0 ±40.8	58.6 ±53.0	0.49	0.49	2.13	0.15	0.69	0.41	1/97
Mean %PPI across three SOA conditions	50.4 ±14.4	30.8 ±28.4	47.9 ±17.4	49.3 ±18.4	44.8 ±21.1	4.29	0.04*	3.47	0.07 ^(*)	5.30	0.02*	1/95

Legend:

Mean values (±SD) are shown. Univariate ANOVAs (for %PPI: ANCOVA with gender and smoking status as covariates) and for the frequency data χ^2 -tests were computed revealing potential differences in the distribution of the shown variables between the genotype groups; * $p < .05$, ^(*) $p < .1$.

Table 2. Demographic and psychophysiological data of the London sample grouped according to *ANKK1* Taq1A and *DRD2* rs7122454 genotype.

<i>ANKK1</i> Taq1A	<i>A2A2</i>		<i>A1</i>		Total	<i>ANKK1</i> Taq1A		<i>DRD2</i> rs7122454		<i>ANKK1</i> Taq1A x <i>DRD2</i> rs7122454		
<i>DRD2</i> rs7122454	CC	CG/GG	CC	CG/GG		F/ χ^2	p	F/ χ^2	p	F/ χ^2	p	df/df _{er}
N	48 (50%)	15 (16%)	27 (28%)	6 (6%)	96 (100%)							
Age, years	26.4±6.0	25.0±6.0	26.7±6.3	25.0±1.9	26.2±5.8	0.01	0.92	0.91	0.34	0.01	0.92	1/93
Men, %	45.8	66.7	39.3	66.7	48.5	0.49	0.53	4.48	0.05*			1
Smokers, %	16.7	33.3	33.3	33.3	25.0	2.29	0.14	0.46	0.56			1
Years of Education	16.9±3.4	17.5±3.3	17.5±4.2	19.0±2.8	17.3±3.6	1.12	0.29	1.12	0.29	0.23	0.63	1/93
First Block, Magnitude of PA Trials (Arbitrary Units)	670.9±371.4	876.2±523.1	691.6±369.0	583.3±273.1	703.2±394.7	1.65	0.20	0.21	0.65	2.20	0.14	1/93
Mean Magnitude of PA Trials (Arbitrary Units)	528.2±346.2	710.4±447.2	584.6±353.9	488.5±180.4	570.2±359.4	0.23	0.64	0.24	0.63	1.27	0.26	1/93
Mean Magnitude of PP30 Trials (Arbitrary Units)	472.9 ± 332.2	687.0 ± 526.4	484.1 ± 322.9	399.3 ± 131.3	495.3 ± 352.1	0.97	0.33	0.37	0.55	1.29	0.26	1/93
Mean Magnitude of PP60 Trials (Arbitrary Units)	343.6 ± 218.1	504.3 ± 394.3	322.2 ± 226.3	264.1 ± 132.5	350.0 ± 247.1	2.62	0.11	0.31	0.58	1.61	0.21	1/93
Mean Magnitude of PP120 Trials (Arbitrary Units)	288.8 ± 202.8	425.3 ± 327.7	312.1 ± 249.0	208.4 ± 80.2	303.4 ± 224.5	1.71	0.19	0.06	0.80	2.15	0.15	1/93
Mean %PPI across three SOA conditions	26.2 ± 19.3	26.8 ± 16.0	38.0 ± 13.2	39.5 ± 11.4	30.4 ± 17.6	7.24	0.01*	0.61	0.44	0.07	0.80	1/90

Legend:

Mean values (±SD) are shown. Univariate ANOVAs (for PPI: ANCOVA with gender and smoking status as covariates) and for the frequency data χ^2 -tests were computed revealing potential differences in the distribution of the shown variables between the genotype groups; * $p < .05$.

Titles and legends to figures

Figure 1. The association of genotype with PPI in the Munich sample.

Legend:

Three different SOAs between prepulse and pulse were used: 30, 60 and 120msec.

Mean PPI (+ SEM) across all participants of the Munich sample and grouped according to the *DRD2* rs7122454 SNP / *ANKK1* Taq1A genotype is depicted (post-hoc t-tests between genotype groups: * $p < .05$; *** $p < .001$; Bonferroni-corrected). Rs7122454 genotype is shown nested within rs1800497 genotype groups.

Figure 2. The association of genotype with PPI in the London sample.

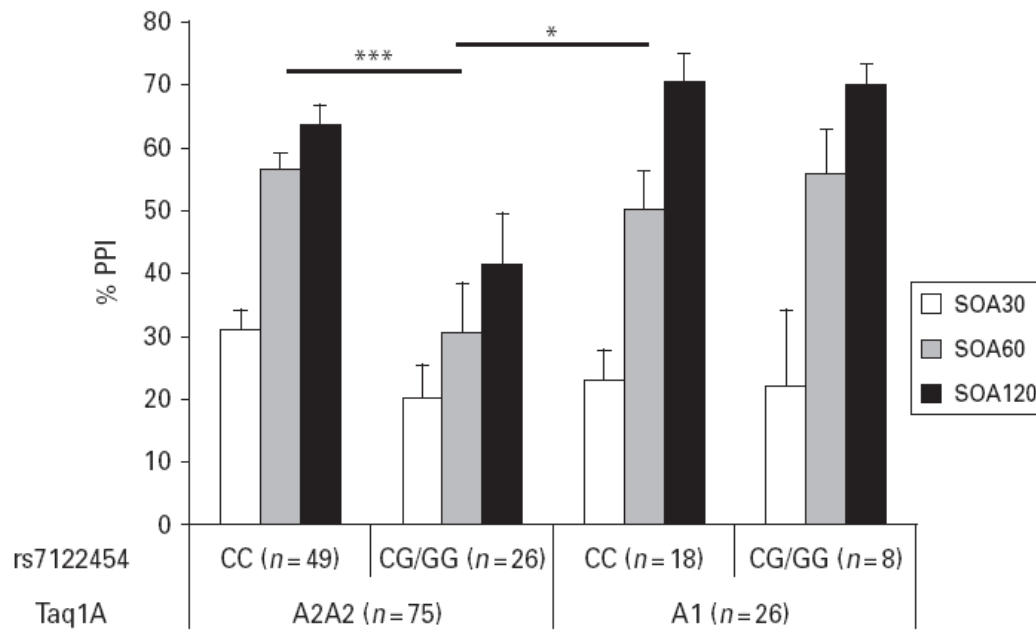
Legend:

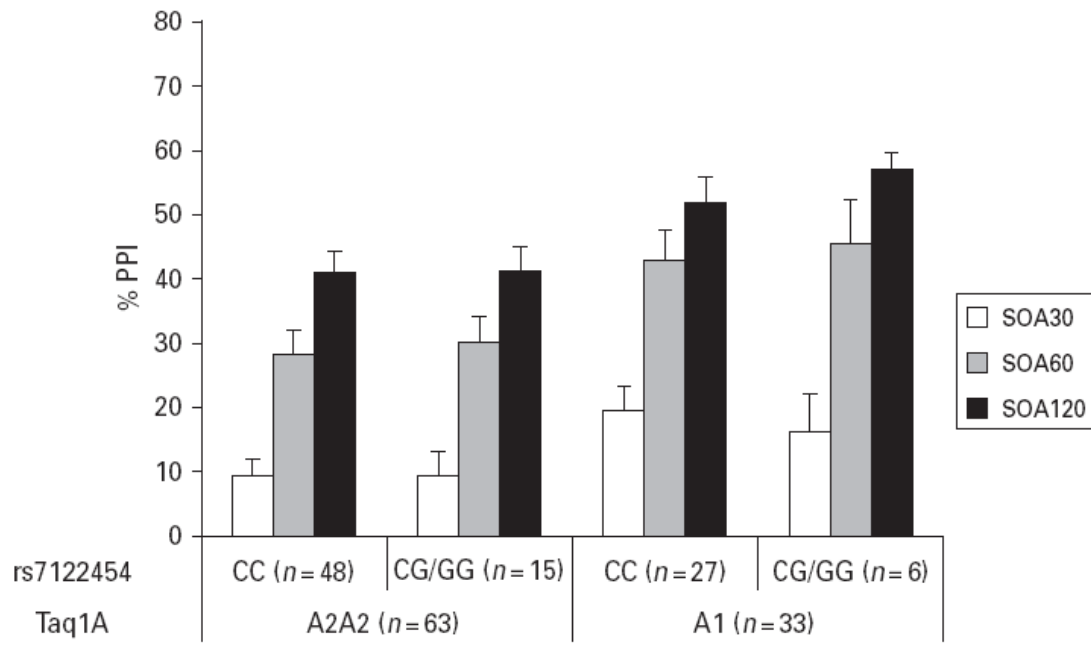
Three different SOAs between prepulse and pulse were used: 30, 60 and 120msec.

Mean PPI (+ SEM) across all participants of the London sample and separated according to the *DRD2* rs7122454 SNP / *ANKK1* Taq1A genotype is shown.

Rs7122454 genotype is shown nested within Taq1A genotype groups. Taq1A

A2A2 allele carrier exhibit significantly reduced PPI compared to A1 allele carriers ($p < .01$).

**Figure 1**

**Figure 2**